

The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (<http://www.aquaculturehub.org>) members and registered individuals and institutions. Please visit our website (<http://siamb.org.il>) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief

Dan Mires

Editorial Board

Sheenan Harpaz	Agricultural Research Organization Beit Dagan, Israel
Zvi Yaron	Dept. of Zoology Tel Aviv University Tel Aviv, Israel
Angelo Colorni	National Center for Mariculture, IOLR Eilat, Israel
Rina Chakrabarti	Aqua Research Lab Dept. of Zoology University of Delhi
Ingrid Lupatsch	Swansea University Singleton Park, Swansea, UK
Jaap van Rijn	The Hebrew University Faculty of Agriculture Israel
Spencer Malecha	Dept. of Human Nutrition, Food and Animal Sciences University of Hawaii
Daniel Golani	The Hebrew University of Jerusalem Jerusalem, Israel
Emilio Tibaldi	Udine University Udine, Italy

Copy Editor

Ellen Rosenberg

Published under auspices of
**The Society of Israeli Aquaculture and
Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library**

and
**University of Hawaii Aquaculture
Program** in association with
AquacultureHub

<http://www.aquaculturehub.org>



UNIVERSITY
of HAWAII
MĀNOA
LIBRARY



AquacultureHub
educate • learn • share • engage

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL

Phone: + 972 52 3965809

<http://siamb.org.il>

Aspects of Comparative Genomics in the Gilthead Sea Bream, *Sparus auratus*

Georgios Kotoulas* and the BRIDGEMAP Consortium**

Hellenic Centre for Marine Research, Heraklion, Crete, Greece

Key words: gilthead sea bream, radiation hybrid mapping, linkage mapping, QTL mapping, comparative genomics, co-expressed genes, selective breeding, aquaculture genomics

Extended Abstract

Genome projects for new fish species are found in a transitional period during which resource development and meaningful comparative genomics analysis or use of any other comparative approach must be done in parallel. In new species, full sequencing is still prohibitive due to the high cost of vertebrate genome sequencing and the fact that present technology is time consuming. However, in the case of the few model species whose research is pioneering and where huge progress has been made on developmental, physiological, behavioral, and genomic levels, knowledge integration is in a process of rapid change. These reasons may explain why a general strategy to facilitate the choice of target species and the kind of developments needed has not yet been adopted. In such an environment, BRIDGEMAP, the European gilthead seabream genome project, was completed in December 2005. This was one of the first genome projects for a non-model fish of aquaculture interest.

BRIDGEMAP was carried out in close collaboration with another European project, BASSMAP, for the genetic linkage mapping of the European sea bass, *Dicentrarchus labrax* (<http://www.bassmap.org/>). Some parallel projects and direct or indirect follow-up projects of BRIDGEMAP, such as AQUAFIRST (<http://aquafirst.vitamib.com/>), are currently running,

while other independent aquaculture genomics projects are on-going in an internationally effervescent genomics environment. This has led to the need for coordination of related aquaculture genomics projects. One is AQUAFUNC (http://ec.europa.eu/research/fp6/ssp/aquafunc_en.htm), the main objective of which is the integration and optimal use of the overall knowledge and functional genomics resources arising from the European Framework programs 5 and 6.

However, despite existing genome sequencing projects, the overall lack of functional information is huge. Therefore, comparative genomics is increasingly applied as a shortcut to link sequence, syntenies, and gene order information, and function. In addition, it constitutes a framework for integration of diverse types of biological data including population genetics, QTL, and expression data. As such, comparative genomics may greatly accelerate the process of linking genes to phenotypes, which may result in a large range of applications.

For organisms in which the complete genome is available, comparative approaches have been very efficient. Nevertheless, more species amenable to such analysis are needed to serve as operational stepping stones between genomes. Economically important fish species are priority candidates due to their

* Tel.: +30-81-2337852; fax: +30-81-2337820; e-mail: kotoulas@her.hcmr.gr

** <http://www.bridgemap.tuc.gr>

socio-economic interest and the considerable information produced on aspects of their husbandry, physiology, biology, and pathology. For these species, however, comparative genome studies are difficult due to a huge lack of molecular resources.

Brief Review of Results

Gilthead sea bream (*Sparus auratus*), a key species of Mediterranean aquaculture, belongs to the species-rich Sparidae family, Perciformes order. From a phylogenetic viewpoint, this order is close to *Fugu rubripes* and *Tetraodon nigroviridis* which have fully sequenced genomes. It is even closer to medaka (*Oryzias latipes*), whose draft genome sequence can be accessed at <http://dolphin.lab.nig.ac.jp/medaka/> and which is a very elegant research model for functional analysis and mutation mapping (Akihiro et al., 2003; Henrich et al., 2004). It is also close to the zebrafish, *Danio rerio* (draft sequence available at http://www.sanger.ac.uk/Projects/D_rerio/), that was the initial target for the comparative genomics of sea bream (Glaser, 2005; Dahm and Geisler, 2006). Contrary to the traditional relationships between model fish species and fish of the Perciformes order such as the gilthead sea bream, Steinke et al. (2006) showed that medaka (Beloniformes), and cichlids (Perciformes) are more closely related to each other than to pufferfish (Tetraodontiformes).

The gilthead sea bream resources and information comprise a genetic linkage map based on about 200 markers (Franch et al., 2006), a radiation hybrid panel that allows easy mapping of thousands of markers and genes (Senger et al., 2006), a radiation hybrid map comprising about 1000 molecular markers and genes (Senger et al., 2006; Sarropoulou, unpublished), cDNA libraries and expressed sequence tags (ESTs) of different tissues (Louro and Power, 2005), polymorphic microsatellite markers (Brown et al., 2005; Franch et al., 2006), population genetic-genomics analysis of environmental and aquaculture samples at the species range for stock structure analysis and traceability (Tsalavouta et al., in prep.; Louro and Power,

in prep.), optimized protocols for chromosome set manipulation for mito-gynogenesis and mito-androgenesis (Gorshkov et al., 1998), and a high quality 6X coverage BAC library. In addition, a partial QTL project for weight at commercial size is in progress, parallel to a breeding program based on parental identification after mass spawning (Batargias et al., in prep.).

Comparative analysis has been conducted both *in silico* and experimentally. It comprises comparative mapping using the recombination independent radiation hybrid map and the genetic linkage map. The radiation hybrid was mainly anchored to *Tetraodon* on the basis of expressed sequences and revealed considerable synteny conservation with *Tetraodon* where, in some cases, gene order was conserved within long syntenic groups (Senger et al., 2006; Sarropoulou et al., unpublished). Surprisingly, the linkage map could be anchored to the *Tetraodon* map, as a considerable number of microsatellites gave significant hits to the *Tetraodon* genome based on analysis of their flanking regions (Franch et al., 2006), very similar to the case of another Perciformes species, *Cottus gobio* (Stemshorn et al., 2005). For the gilthead sea bream linkage map, males derived recombination events in some linkage groups less frequently than females, while in other chromosomes the reverse occurred, making sex specific maps that differed for different linkage groups. Nevertheless, at the whole genome level, the length of the male and female maps did not differ significantly. Given that sea bream is a sequential hermaphrodite, this result is both surprising and interesting. Comparison of the radiation hybrid map and the linkage map gave fairly consistent results. The distribution of recombination frequency across the genome could be mapped at the level of resolution of the radiation hybrid map, since it is independent of recombination and closer to a physical map (Sarropoulou, unpublished).

An experimental shortcut to comparative mapping has been the use of cross species amplification of mapped microsatellite markers, an approach that may lead to comparative linkage maps. We applied this approach at an

exploratory level with considerable success within Sparid species (Brown et al., 2005; Franch et al., 2006) and between gilthead sea bream and the European sea bass, *D. labrax* (Chistiakov et al., 2005; Tsigenopoulos et al., in prep.).

The use of a comparative genomics framework for integration of different aspects of biological research was exemplified by mapping genes that were co-expressed in microarray analysis on the radiation hybrid map, an approach we are following by increasing the number of mapped genes for genes with available expression analysis (Sarropoulou et al., 2005).

The above results reveal the high potential of combining genetic approaches based on polymorphic markers (such as QTL analysis) with candidate gene approaches (by using SNP information) for the establishment of gene-to-phenotype links and achievement of efficient selective breeding programs. The produced resources make the gilthead sea bream a good target species for genomic applications in aquaculture, as well as for assessing aspects of the development and biology of the species and its adaptation to different environmental conditions including domestication.

References

- Akihiro S., Himmelbauer H., Mitani H., Furutani-Seiki M., Wittbrodt J. and M. Schartl**, 2003. Fish genomes flyin. *EMBO Rep.*, 4(2):121–125.
- Brown R.C., Tsalavouta M., Terzoglou V., Magoulas A. and B.J. Mcandrew**, 2005. Additional microsatellites for *Sparus aurata* and cross-species amplification within the Sparidae family. *Mol. Ecol. Notes*, 5:605.
- Chistiakov D., Hellemans B., Haley C., Law A., Tsigenopoulos C.S., Kotoulas G., Bertotto D., Libertini A. and F.A.M. Volckaert**, 2005. A microsatellite linkage map of the European seabass *Dicentrarchus labrax* L. *Genetics*, 170:1821–1826.
- Dahm R. and R. Geisler**, 2006. Learning from small fry: the zebrafish as a genetic model organism for aquaculture fish species. *Mar. Biotechnol.*, 8(4):329–345.
- Dirk S., Salzburger W. and A. Meyer**, 2006. Novel relationships among ten fish model species revealed based on a phylogenomic analysis using ESTs. *J. Mol. Evol.*, 62(6): 772–784.
- Franch R., Louro B., Tsalavouta M., Chatziplis D., Tsigenopoulos C.S., Sarropoulou E., Antonello J., Magoulas A., Patarnello T., Power D.M., Kotoulas G. and L. Bargelloni**, 2006. A genetic linkage map of the hermaphrodite teleost fish *Sparus aurata* L. *Genetics*, in press.
- Glaser V.**, 2005. Investigator profile: an interview with Robert Geisler, Ph.D. *Zebrafish*, 2(4):237–241.
- Gorshkov S., Gorshkova G., Hadani A., Gordin H. and W.R. Knibb**, 1998. Chromosome set manipulations and hybridization experiments in gilthead seabream (*Sparus aurata*). I. Induced gynogenesis and intergeneric hybridization using males of the red seabream (*Pagrus major*). *Isr. J. Aquac. - Bamidgeh*, 50:99–110.
- Henrich T., Ramialison M., Segerdell E., Westerfield M., Furutani-Seiki M., Wittbrodt J. and H. Kondoh**, 2004. GSD: a genetic screen database. *Mech. Dev.*, 121:959–963.
- Louro B.E.P. and D.M. Power**, 2005. Transcriptome analysis of the gilthead sea bream (*Sparus auratus*) pituitary gland: type I markers for molecular genetics. *Rev. Portuguesa de Zootecnia*, 2:91–104.
- Sarropoulou E., Kotoulas G., Power D.M. and R. Geisler**, 2005. Gene expression profiling of the gilthead sea bream during early development and detection of stress-related genes by the application of cDNA microarray technology. *Physiol. Genom.*, 23:182–191.
- Senger F., Priat C., Hitte C., Sarropoulou E., Franch R., Geisler R., Bargelloni L., Power D.M. and F. Galibert**, 2006. The first radiation hybrid map of a perch-like fish: the gilthead seabream (*Sparus aurata* L.). *Genomics*, 87: 793–800.
- Stemshorn K.C., Nolte A.W. and D. Tautz**, 2005. A genetic map of *Cottus gobio* (Pisces, Teleostei) based on microsatellites can be linked to the physical map of *Tetraodon nigroviridis*. *J. Evol. Biol.*, 18:1619–1624.

Genetic Basis of Sex Determination in Fishes: Searching for Master Key Regulator Genes in the Sex Determination Pathway of Tilapias*

Andrey Shirak^{1**}, Eyal Seroussi¹, Noam Zilberman¹, Gideon Hulata¹, Micha Ron¹,
Avner Cnaani² and Thomas D. Kocher²

¹ Institute of Animal Science, Agricultural Research Organization, Volcani Center,
P.O. Box 6, Bet Dagan 50250 Israel

² Hubbard Center for Genome Studies, University of New Hampshire, Durham,
NH 03824, USA

Key words: genome mapping, candidate genes, sex determination, tilapia, *Oreochromis* spp.

Abstract

Recent results show a striking similarity in the dynamics of expression of gonad differentiation regulators in zebrafish and mammals. Candidates for the role of master key regulators (MKRs) in tilapia were selected, based on three concepts established in the literature for non-mammal species. These three concepts are: (a) MKRs are DM-proteins or their close downstream/upstream partners in the sex determination pathway, (b) MKRs are genes working just upstream of aromatase in the sex determination pathway, or the aromatase itself, and (c) MKRs are homologs of mammal genes which are close partners of SRY ("missing" in non-mammal vertebrates), mostly belonging to the SOX family. Coding sequences of putative genes were searched in cichlid (TIGR) and general (NCBI) databases, and in the tilapia gonad EST library (RBEST). Primers in two adjacent exons were designed, based on predicted exon-intron boundaries for each of 11 selected genes. Amplified segments of the targeted genes in two purebred tilapia species were sequenced. Seven SSLP and four SNP-based markers were identified in the candidate genes for MKRs of sex determination and mapped to the tilapia genetic map using genotype data of 76-90 individuals of the F₂ mapping family. The mapping positions of the selected genes relative to previously reported QTL regions for sex determination are discussed.

* The full paper: *Amh* and *Dmrt2* Genes Map to Tilapia (*Oreochromis* spp.) Linkage Group 23 within QTL Regions for Sex Determination, by Andrey Shirak, Eyal Seroussi, Avner Cnaani, Aimee E. Howe, Raisa Domokhovskiy, Noam Zilberman, Thomas D. Kocher, Gideon Hulata and Micha Ron has been accepted for publication by *Genetics*.

** Corresponding author. E-mail: shirak2@bezeqint.net